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TITLE: "Development of Nanomedicines for Treatment of Posttraumatic Osteoarthritis"

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14. ABSTRACT During this funding period, <i>N</i> -(2-hydroxypropyl)methacrylamide (HPMA) copolymers dexamethasone conjugates were synthesized and labeled with near-infrared dye IRDye CW 800 or Alexa Fluor 647 (P-Dex-IRDye or P-Dex-Alexa). Mice with surgical destabilization of the medial meniscus (DMM) were established as an osteoarthritis model for our evaluation. P-Dex-IRDye and P-Dex-Alexa were given to the DMM mice via intraarticular injection. Optical imaging data suggest that the DMM joints were able to retain the injected P-Dex-IRDye. The treated joints have been isolated for immunohistochemistry and flow cytometry analysis to identify cell phenotypes that internalize the P-Dex-Alexa.						
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1. INTRODUCTION:

The current proposal aims to design nanomedicines to selectively target cells within the osteoarthritic joint, and to exploit this to enhance the delivery, retention, and efficacy of therapeutic agents. Osteoarthritis and post-traumatic osteoarthritis (OA/PTOA) are the leading cause of disability and loss of work capacity in the adult population, yet there remains an unmet clinical need for effective therapies to prevent disease progression. The proposed studies are designed to investigate novel nanomedicine mechanisms of pharmacotherapy for OA, using a combination of in vitro and in vivo studies.

2. KEYWORDS:

Nanomedicine, PTOA, DMM, osteoarthritis, prodrug, glucocorticoid, dexamethasone, HPMA copolymer

3. ACCOMPLISHMENTS:

What were the major goals of the project?

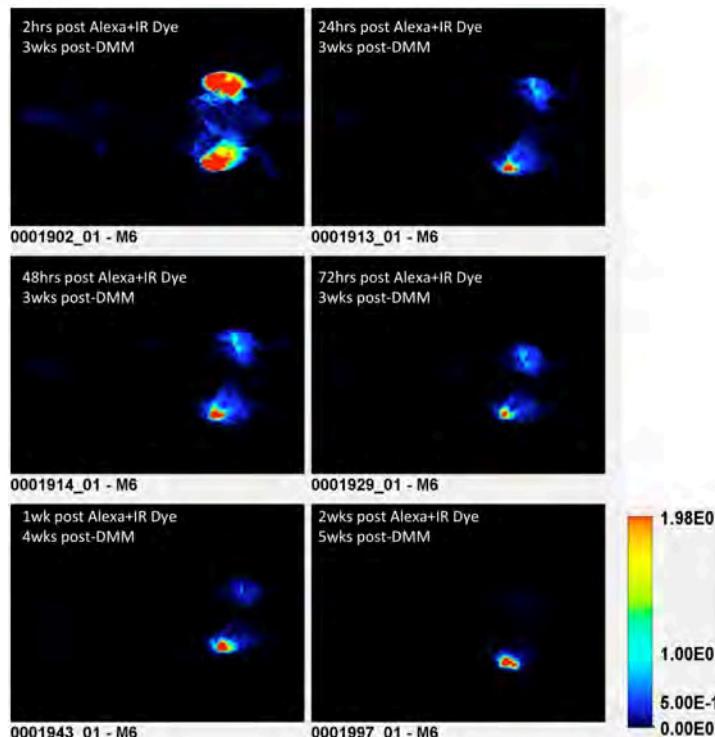
- Task 1. Identify patterns of cellular uptake and retention of nanomedicines in the knee joints of mice with OA induced by destabilization of the medial meniscus (DMM). Months 1-12.
- Task 2. Optimize structural parameters for delivery and sustained therapeutic efficacy of Dexamethasone and BMS 345541 prodrugs. Months 13-18.

What was accomplished under these goals?

- Synthesis and characterization of IRDye-labeled and AlexaFluor488-labeled HPMA copolymers. Months 1-2.
To synthesize IRDye and Alexa-labeled HPMA copolymers, *N*-(2-Hydroxypropyl)methacrylamide (HPMA), *N*-(3-aminopropyl)methacrylamide (APMA), 2,2'-azobisisobutyronitrile (AIBN) and S,S'-bis(α , α' -dimethyl- α'' -acetic acid) trithiocarbonate were dissolved in methanol, transferred into an ampule, purged with argon for 5 min and then flame-sealed. It was maintained at 45 °C for 48 hr for polymerization, after which the polymer product was purified by re-precipitation and LH-20 column. The content of the primary amine in the copolymer was determined by ninhydrin assay. The polymer precursors were then incubated together with NHS esters of IRDye or Alexa 488 (in the presence of DIPEA) at room temperature overnight to introduce the fluorescent labels. The labeled copolymers were further purified by LH-20 column and their molecular weight (MW) and polydispersity (PDI) were determined by FPLC using HPMA homopolymer calibration. The content of labels were determined by UV/Vis spectrophotometry.
- Surgical induction of DMM in mice. Months 1-4.
DMM surgery was performed on a total of 15 mice. In each mouse, the right knee was subjected to transection of the medial tibial ligament, and the left knee was unoperated (control).

- Intraarticular injection of HPMA copolymers, optical imaging to define patterns of retention within the joints. Months 3-8.

Five mice with DMM surgery were administered a mixture of 0.5mg P-IRDye + 0.5mg P-Alexa in a total volume of 5 microliters of PBS into each knee by intraarticular injection at three weeks post surgery. Successful introduction of the HPMA copolymers into the joint space was confirmed by optical imaging 2 hours after injection. This showed strong IRDye signals in the injected knees. Two additional mice were injected with PBS (controls). An additional 5 mice and 3 controls were treated with HPMA copolymers in the same way at 4 weeks post surgery. All mice were optically imaged over 2 weeks. This analysis showed that the intraarticularly injected HPMA copolymers were retained within the joint space over the time course, with signal still present at 2 weeks after injection (see Figure below). Both operated and unoperated knees showed retention of HPMA copolymers over 2 weeks, although some variations in the intensity of the relative signals were noted.



- Recovery of joint tissues and cells, establishment of disease progression and cellular localization using faxitron, microCT, histological and immunohistological analyses, and flow cytometry. Months 8-12.

Following optical imaging at 2 weeks after intra-articular injection of the HPMA copolymers, the mice were sacrificed and the knee joints were fixed with paraformaldehyde, decalcified with EDTA, paraffin-embedded, and sectioned. Processing of the sections for histological scoring (Safranin-O ad fast green staining) and for fluorescent microscopy to localize the P-Alexa are currently ongoing.

What opportunities for training and professional development has the project provided?

How were the results disseminated to communities of interest?

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

During the next funding period, we will complete the histological and fluorescent microscopy studies from Task 1 and focus on experiments proposed in Task 2. Using in vitro cell culture systems, we will optimize structural parameters for delivery and sustained therapeutic efficacy of dexamethasone and BMS 345541 prodrugs

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Actual or anticipated problems or delays and actions or plans to resolve them

The histological, fluorescent microscopic and immunocytochemical studies on the DMM mice are still ongoing, and will be completed within the next months. The flow cytometry approaches have been delayed due to limitations of the amount of retrieved tissue to work with, and to allow us to focus upon the histological analysis, which should provide comprehensive data upon the cellular localization of the injected HPMA.

6. PRODUCTS:

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Dong Wang
Project Role:	Principle investigator
Person month worked:	1.2
Contribution to Project:	Dr. Wang is responsible for overseeing the entire project, especially the synthesis and characterization of the various

	HPMA copolymers, and HPMA copolymer-drug conjugates used in the study.
Funding Support:	N/A
Name:	P. Edward Purdue
Project Role:	Principle investigator at the subcontract site (HSS)
Person month worked:	1.2
Contribution to Project:	Dr. Purdue directed and helped oversee experiments involving the murine OA model, which were established within Dr. Mary Goldring's Research Laboratory, and the processing of tissue for histological analysis.
Funding Support:	N/A
Name:	Mary B. Goldring
Project Role:	Co-investigator at the subcontract site (HSS)
Person month worked:	0.6
Contribution to Project:	Dr. M. Goldring participated in experimental design and troubleshooting, analysis of data and interpretation of results.
Funding Support:	N/A
Name:	Steven R. Goldring
Project Role:	Co-investigator at the subcontract site (HSS)
Person month worked:	1.2
Contribution to Project:	Dr. S. Goldring participated in experimental design and troubleshooting, analysis of data and interpretation of results.
Funding Support:	N/A
Name:	Aaron Daluiski
Project Role:	Co-investigator at the subcontract site (HSS)
Person month worked:	0.6
Contribution to Project:	Dr. Daluiski is the Co-I at HSS. He assisted in carrying out the mouse OA model and evaluating the outcomes of the surgeries.
Funding Support:	N/A
Name:	Jianbo Wu
Project Role:	Postdoc with Dr. Wang
Person month worked:	3
Contribution to Project:	Dr. Wu focused on the synthesis and characterization of polymer prodrugs.
Funding Support:	N/A
Name:	Kirstey Culley
Project Role:	Postdoc with Dr. M. Goldring
Person month worked:	1.8
Contribution to Project:	Dr. Culley established DMM model and performed the

Funding Support: N/A intraarticular injections on the mice and tissue retrieval.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

New funding

Grant title: Development of dentotropic tooth whitening agents
Funding agency: Johnson & Johnson Consumer Companies, Inc.
Duration: 01/19/2015-02/18/2016
PI: Dong Wang

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS: None

9. APPENDICES: None